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Enzymatic Approach to Biotransformation

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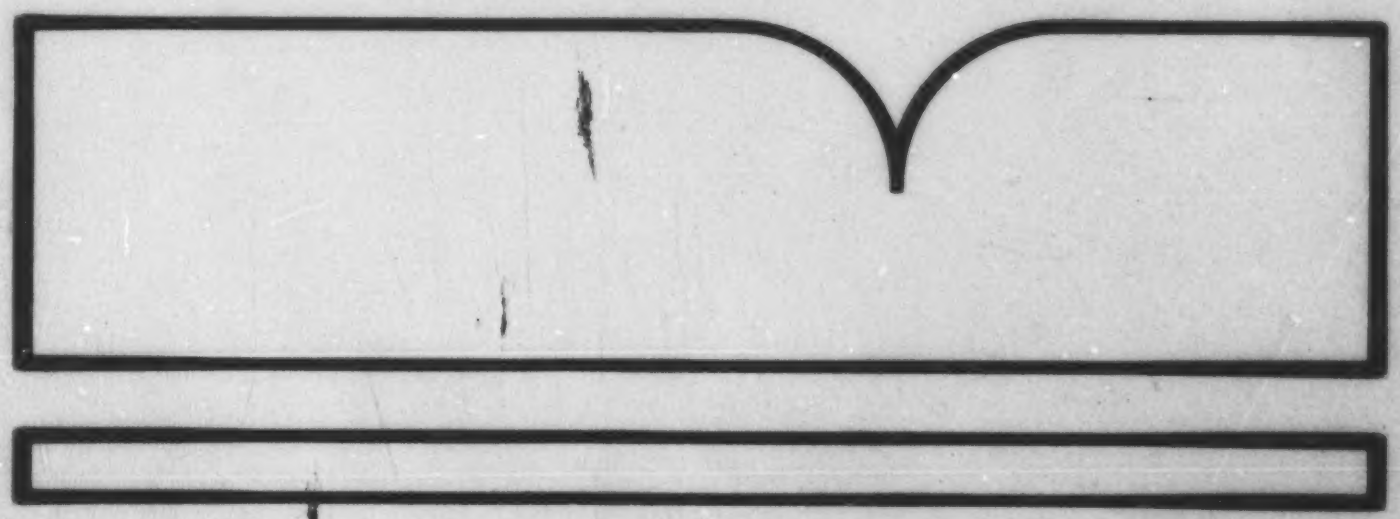

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An overall scheme for the biotransformation of organic compounds by routes other than hydrolysis of possible ester bonds is presented. Both phase I (oxidative) and phase II (conjugative) biotransformation reactions are discussed on an enzyme basis. Whenever possible pesticides are used as examples of the mechanisms by which enzyme activities alter chemical structure (biotransformation) as well as the ways in which chemicals themselves can effect enzyme activities.

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An Enzymatic Approach to Biotransformation

Kirk T. Kitchin

ABSTRACT

An overall scheme for the biotransformation of organic compounds by routes other than hydrolysis of possible ester bonds is presented. Both phase I (oxidative) and phase II (conjugative) biotransformation reactions are discussed on an enzyme by enzyme basis. Whenever possible pesticides are used as examples of the mechanisms by which enzyme activities alter chemical structure (biotransformation) as well as the ways in which chemicals themselves can effect enzyme activities.

Key words: Enzyme induction - Drug metabolizing enzymes - Glutathione depletion - Covalent binding.

A simplified overall scheme of hepatic pesticide biotransformation and its effects on pharmacokinetics and pharmacodynamics are presented in Figure 1. Pesticide pharmacokinetics are altered by phase I oxidative drug metabolism enzymes which results in the increasing polarity (water solubility) of pesticide parent compounds and shorter biological half lives. Phase II conjugation reactions can unite either the parent or the modified pesticide moiety with highly water soluble molecules such as UDP-glucuronic acid or glutathione. Thus, biotransformation results in more polar, readily excretable chemical forms (Table 1), which may be eliminated from the body via urinary or biliary routes. Generally, biotransformation produces less toxic metabolite molecules, however, this is not always the case.

Biotransformation of pesticides to reactive intermediates which covalently bind to tissue macromolecules can increase the biological half life of the attached pesticide moiety. If the pesticide metabolite is bound to a critical cellular site such as DNA or neurologically important esterases, oncogenic or neurotoxic sequelae may be initiated (Table 1).

Experimental Biology Division, Health Effects Research Laboratory, Research Triangle Park, NC 27711.

Parathion is metabolized to paraoxon which is the active acetylcholinesterase inhibitor. The hepatic cytochrome P-450 monooxygenase system catalyses this activation or toxification process and thus parathion is a useful insecticide. Many compounds, including non pesticides, are metabolized prior to excretion and occasionally highly reactive intermediates are formed. These chemical intermediates may be present in low cellular concentrations and possess short half lives but still be responsible for much of the toxicity observed after administration of the parent compound. Two examples of this are the metabolism of benzo[a]pyrene to a highly carcinogenic diolepoxide metabolite (1) and the metabolism of bromobenzene to the hepatoxic species 3,4-epoxybromobenzene (2). Some reactive metabolites covalently bind to DNA, protein or other cellular macromolecules and may initiate genotoxic responses. In carcinogenesis (3) and mutagenesis (4, 5), evidence is strong that reactive intermediates are a frequent cause of this genotoxicity. Recently, evidence has been presented that some teratogenesis depends on prior biotransformation by the cytochrome P-450 system (6). An example of biotransformation to a less toxic form is the 4-hydroxylation of carbaryl and the subsequent glucuronidation and excretion of the metabolized pesticide.

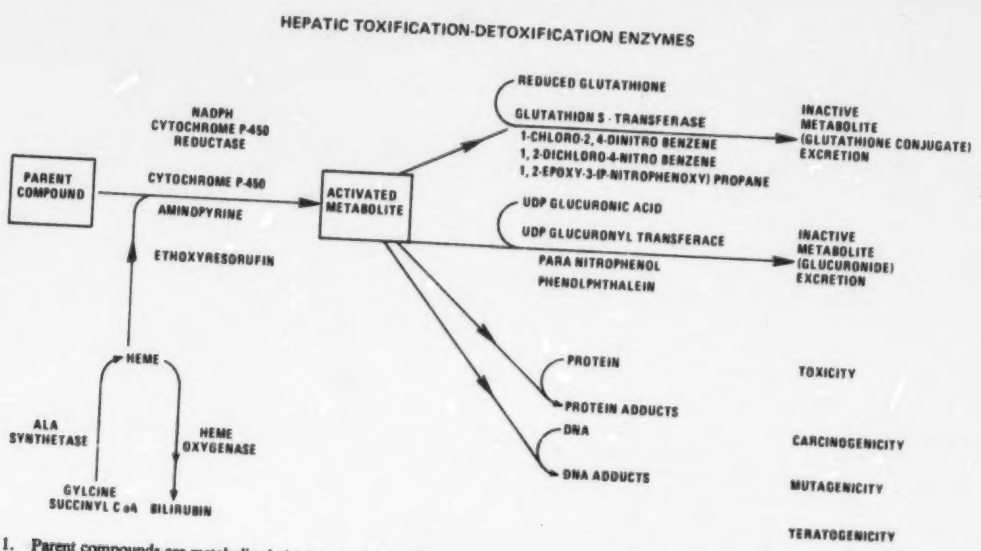

FIG. 1. Parent compounds are metabolized via the cytochrome P-450 monooxygenase system. Metabolites may be detoxified via the glutathione-S-transferase system or the glucuronidation system. Alternatively an activated metabolite may form adducts with important tissue macromolecules such as protein or DNA. This covalent binding can lead to toxicity, carcinogenicity, mutagenicity or teratogencity. Representative substrates for the enzymes are written below the enzyme name while cofactors are written just above it. (Reprinted from 87.)

Assessment of the importance of biotransformation in pesticide toxicology is made more difficult by the presence of multiple forms of several of these enzymes such as cytochrome P-450 (7-11), glutathione S-transferase (12-14), and UDP-glucuronyltransferase (15, 16). Different forms of enzymes may be induced by different inducing molecules (7, 10, 11). For any individual form of an enzyme, the substrate specifity may or may not be similar to the other forms of the same enzyme (7, 9, 11). Chemicals may affect enzyme activities by induction, repression, cofactor depletion, inactivation or inhibition (Table 2). The enzymatic elements of the scheme presented in Figure 1 will be discussed sequentially in greater detail below.

TABLE 1. Ways in which enzyme activities may affect chemicals

	Example
Pharmacokinetic	
Decrease biological half life of molecule via biotransformation.	Lindane metabolism to 2,4,6 trichlorophenol*
Increase biological half life of molecule via biotransformation and subsequent binding.	Binding of 7,8-dihydroxy-9,10-epoxybenzo[a]pyrene to DNA.
Pharmacodynamic	
Biotransformation to a less toxic form.	Paraoxon metabolism to p-nitrophenol.
Biotransformation to a more toxic form.	Benzo[a]pyrene metabolism to 7,8-dihydroxy-9,10-epoxybenzo[a]pyrene.
	Parathion metabolism to paraoxon

* In addition to those given here many other metabolities of lindane and benzo[a]pyrene are known.

TABLE 2. Ways in which chemicals may affect enzyme activities

Effect	Example
Induction	DDT increases cytochrome P-450 content.
Repression	Heme represses ALA synthetase.
Cofactor depletion	Bromobenzene depletes hepatic glutathione content
Inactivation	Allylisopropylacetamide decreases cytochrome P-450 content (86).
Inhibition	Carbaryl inhibits acetylcholinesterase.

HEME SYNTHESIS AND DEGRADATION

The rate limiting step of hepatic heme synthesis, δ-aminolevulinic acid synthetase (ALAS), is an inducible and repressable mitochondrial enzyme (17). It is thought to be under physiologic control by endogenous repression by heme, the end product of the biochemical pathway (17, 18). Administration of certain chlorinated hydrocarbons such as hexachlorobenzene induces ALAS and causes and excess accumulation of porphyrins (19). Deposition of porphyrins in the skin can lead to phototoxicity and skin lesions in experimental animals and man (19, 20). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), a synthesis contaminant of the herbicide 2,4,5-T, can also induce ALAS (21) and cause porphyria in toxic doses.

Some ALAS inducers also increase hepatic cytochrome P-450 levels. Since about 40 % of the synthesized hepatic heme is required to form hepatic microsomal cytochrome P-450 (22), the coordinate induction of ALAS and cytochrome P-450 to form required committed heme would seem to make teleological sense. For example, phenobarbital, benzo[a]pyrene and TCDD all induce ALAS and cytochrome P-450 levels at high doses. However, in some instances substantial cytochrome P-450 induction has been observed without any concommitant induction (derepression) of ALAS caused by lowered endogenous heme levels (23). This lack of coordinate induction is further supported by the observation that both $CoCl_2$ and allylisopropylacetamide administration induce ALAS but actually decrease cytochrome P-450 levels (24, 25).

Microsomal heme oxygenase is an inducible enzyme which catalyzes the conversion of heme to biliverdin from which bilirubin, a toxic physiological intermediate, is formed. This rate limiting step of hepatic heme degradation is inducible by many metal cations (26, 27) and some organometals as well. The best studied heme oxygenase inducers are it's organometal substrate, heme and $CoCl_2$. The organometal pesticides such as maneb or organic arsenic pesticides have not been well investigated with respect to their possible effects on this enzyme system. During the neonatal period many mammals produce more bilirubin via heme oxygenase than they can conjugate via UDP-glucuronyltransferase and the resulting accumulation of bilirubin may prove toxic (*i.e.*, kernictus). The cellular enzymatic levels of ALAS and heme oxygenase are closely related and cyclic oscillations in their enzyme activities can be induced by heme administration (28).

CYTOCHROME P-450

This versatile hemoprotein is found in most organs of mammals and in insects as well. At least 8 forms of this protein exist in rodent liver (7-11). The 2 main forms, cytochrome P-450 and cytochrome P-448, refer to the phenobarbital or 3-methylcholanthrene inducible forms, respectively. Spectral, enzymatic and biochemical methods have been employed to differentiate between various cytochrome P-450 forms. The representative substrates aminopyrine (P-450) and ethyoxyresorufin or benzo[a]pyrene (P-448) can be used as markers for these different forms of these rat hemoproteins (29, 30). Of the various pesticide classes the polychlorinated hydrocarbons and cyclodienes are the most active cytochrome P-450 inducers (Table 3). DDT, aldrin/dieldrin, chlordane, toxaphene, heptachlor, lindane and mirex are all effective inducers. Chlorinated benzenes, biphenyls, dibenzofurans and dibenzodioxins are also inducers. TCDD, the most potent inducer known, can induce hepatic microsomal aryl hydrocarbon hydroxylase activity in doses as low as 0.002 μg/kg and can produce a maximal 40-fold induction in female rats (76).

In general, most cytochrome P-450 inducers are somewhat planar, lipid soluble organic molecules, and may be substrates of cytochrome P-450 system as well. For most of these molecules, no structure activity relationship has been identified and a high degree of lipid solubility appears to be the only requirement for enzyme induction.

Recent studies have identified a high affinity cytosolic receptor which binds potent cytochrome P-448 inducers such as TCDD. This saturatable protein receptor exhibits a structure activity relationship for binding ligands which closely corresponds to the *in vivo* cytochrome P-448 induction pattern (77). After binding the ligand, the cytosolic receptor, or Ah (aromatic hydrocarbon) receptor, translocates to the nucleus and initiates gene expression (78).

In general there are two families of cytochrome P-450 inducers. The less specific type, exemplified by phenobarbital, increases liver weight, microsomal protein, NADPH-cytochrome P-450 reductase, ALA synthetase, cytochrome P-450 content and its associated activities, and some glutathione S-transferase activities. Spectrally, rodent liver cytochrome P-450 induced by phenobarbital is very similar to endogenous rodent cytochrome P-450.

3-Methylcholanthrene or TCDD administration has more specific inductive effects. A cytochrome of dif-

TABLE 3. Induction of microsomal mixed function oxidase enzymes by chlorinated insecticides

Insecticide	Animal	Reference
DDT	Rat	31-44
	Mouse	45-49
	Guinea Pig	50
	Dog	51
	Man	52-54
	Cell Culture	55-56
DDT analogs and related compounds	Rat	32-33, 35, 57-60
	Mouse	45, 46
	Dog	60
	Man	52
Aldrin/Dieldrin	Rat	62-64
	Mouse	62, 65, 66
	Guinea Pig	50
	Pig	62
	Monkey	62
	Man	52
Chlordane	Rat	34, 49, 67-69
	Mouse	48
	Dog	34
	Squirrel Monkey	70,71
Toxaphene	Rat	37
Heptachlor	Rat	63, 72
Lindane	Rat	73, 74
	Man	53
	Guinea Pig	50
Mirex	Mouse	75
	Rat	75

Adapted from Hodgson *et al.* (88).

ferent spectral characteristics (*e.g.*, CO reduced λ max at 448 nm) is perferentially synthesized and enzyme activities like ethoxyresorufin O-deethylase and aryl hydrocarbon hydroxylase are greatly induced. In rat liver the cytochrome P-450, dependent metabolism activities induced by phenobarbital and 3-metylcholanthrene do not overlap to a significant extent.

Another class of pesticides which interact with the cytochrome P-450 system are the methylenedioxyphenyl synergists. A biphasic interaction of these compounds with the cytochrome P-450 monooxygenase system has been observed. First inhibition occurs and then a following phase of cytochrome P-450 enzyme induction is initiated. These insecticides are often used in combination with other insecticides in order to take advantage of their ability to inhibit insect cytochrome P-450 function *in vivo*.

The cytochrome P-450 dependent monooxygenase system requires a flow of electrons obtained from the coupled flavoprotein NADPH-cytochrome P-450 reductase. Ultimately these electrons are derived from the oxidation of NADPH or NADH. The NADPH-cytochrome P-450 reductase system is also inducible. Up to 2-3 fold increases in the activity of this enzyme have been observed after the administration of phenobarbital or chlorinated benzenes. As some investigators have hypothesized that the activity of the reductase enzyme may limit cytochrome P-450 activity (79), coordinate induction of NADPH-cytochrome P-450 reductase activity along with cytochrome P-450 becomes important in altering the overall rates of biotransformation. In studies with the reconstituted monooxygenase system, either the lipid, hemoprotein or reductase component can be made rate limiting (80). *In vitro* incubation of exogenous cytochrome P-450 with microsomal membranes does not always produce higher rates of cytochrome P-450 catalysis (79, 80). This would be expected if the reductase component of the hepatic monooxygenase system is rate limiting.

GLUTATHIONE S-TRANSFERASE

The two most important biological consequences of the interaction of pesticides with glutathione S-transferase are enzyme induction and cofactor depletion. Glutathione S-transferases occur in several different forms (about 6 or more) in cell cytosol and the substrate specificity of these isoenzymes overlap to a greater extent than does the cytochrome P-450 isoenzyme family (12-14). Three substrates frequently employed to assay its activity *in vitro* are 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene and 1,2-epoxy-3-(p-nitrophenoxy)propane. The glutathione S-transferases can be induced about 2 to 3-foid but in general these cytosolic enzymes are less responsive than the cytochrome P-450 system.

This point is quite important for risk assessment. For example, if hepatic toxification reaction rates are increased 4-fold and detoxification rates are increased only 2-fold, theory predicts the steady state concentration of reactive intermediates should be increased. If the tox-

icant does not saturate either enzyme system, the reactive intermediate concentration will increase to a certain limited extent. If detoxification pathways saturate first, substantial accumulation of reactive intermediates, covalent binding, cellular destruction, tissue toxicity and genotoxic sequalae can all result.

Studies with bromobenzene and acetaminophen have developed the concept of glutathione depletion as an important mechanism of part of the toxicity of these compounds. Depletion of greater than 80 % of hepatic glutathione by acetaminophen administration (82-84) results in substantial increases in tissue covalent binding of the compound. Thus, glutathione depletion may underlie the dose response thresholds often observed in pesticide toxicology (85).

Some compounds will induce cytochrome P-450 and cytosolic glutathione S-transferase in a coordinate fashion. The uninduced specific activity of glutathione S-transferase towards certain substrates is much higher than the rate of metabolite production by cytochrome P-450. Liver cytosolic glutathione concentration is approximately 5 mMolar. As the ratio of liver cytosolic/microsomal protein is approximately 5/1, the liver has a large amount of glutathione S-transferase activity available as a detoxification pathway. In addition to enzymatic glutathione conjugation, some nonenzymatic glutathione conjugation may occur as well.

UDP-GLUCURONYLTRANSFERASE

As with several other enzymes mediating biotransformation, multiple forms of UDP-glucuronyltransferase (GT) have been identified (15, 16). Wishart (16) has distinguished between 'late fetal' GT (representative substrate p-nitrophenol) and 'neonatal' GT (representative substrate phenolphthalein) forms on the basis of gluccorticoid induction, zenobiotic induction, smooth/rough endoplasmic reticulum ratios, ontogeny and substrate specificity. The GT isoenzyme forms take their name from the time in which their enzyme activity is at its perinatal peak. Only late fetal GT is highly inducible; neonatal GT is not significantly responsive to environmental chemicals. The late fetal GT isoenzyme tends to conjugate smaller, more planar structures while neonatal GT substrates can be large, bulky molecules. The neonatal GT isoenzyme metabolizes several substrates of physiologic or toxicologic importance such as bilirubin, testosterone, estrogen, chloramphenicol and morphine. Since enzyme neonatal GT is relatively noninducible, this is important to take into consideration in risk evaluation. If bilirubin production is increased several fold while the conjugative neonatal GT activity remains unchanged, toxic levels of bilirubin may result. This is entirely different from a situation where the activity of many phase I and II biotransformation enzymes is increased in a coordinate fashion to a similar extent. Late fetal GT is highly inducible by cytochrome P-448 type inducers, such as 3-methylcholanthrene or TCDD, and thus coordinate induction of cytochrome P-448 and late fetal GT can occur. Few studies on the effects of pesticides on either GT form have been published.

COVALENT BINDING

Covalent binding of toxicants to tissue macromolecules, particularly DNA, have been implicated as a mechanism of carcinogenicity (3) and mutagenicity (4, 5). When radiolabelled carcinogens are given to experimental animal, DNA-, RNA- and protein-bound derivatives are found in target tissues (3). Irregardless of whether they require metabolic activation or not, electrophilicity is a common characteristic of ultimate carcinogens.

Covalent binding of ^{14}C-bromobenzene and ^{14}C-acetaminophen correlates well with hepatic cell death and whole animal toxicity (82-85). Selective induction of various enzymatic pathways or replenishing depleted tissue glutathione stores alters the toxicity of these agents in ways which can be predicted from Figure 1. For example, diethyl maleate pretreatment depletes glutathione and increases the hepatoxicity of acetaminophen (83). Phenobarbital administration has a similar effect via a different mechanism (cytochrome P-450 induction) (82).

Mutagenicity often correlates well, but not perfectly with carcinogenesis. The well known Ames Test has demostrated the utility of this high correlation between mutagenicity and carcinogenicity. The usefulness of this *in vitro* test system has helped promote the development of many other *in vitro* techniques to characterize the potential mutagenicity and carcinogenicity of chemicals. Few or no direct measures of covalent binding to cellular constituents have been proposed as toxicity screens. The cost of radiolabelling each individual proposed pesticide (perhaps in several different atoms) has to be considered as part of the cost of any ^{14}C-pesticide-covalent binding assay.

CONCLUSION

An overall scheme to account for some pesticide biotransformation, pharmacokinetics and pharmacodynamics is presented in Figure 1. As with any simplification, this model will not work well for all com-

TABLE 4. Representative rat hepatic enzyme activities* (nmole/mg protein/min)

	Male	Female	Day 14 Pregnant Female
δ-Aminolevulinic acid synthetase	0.019	0.017	—
Heme oxygenase	0.015	—	—
NADPH-cytochrome P-450 reductase (using cytochrome c as substrate)	120.	107.	116.
Cytochrome P-450 (nmol/mg protein)	1.06	0.85	0.73
Aminopyrine N-demethylase	—	3.94	2.61
Ethoxyresorufin O-deethylase	0.022	—	0.014
Aryl hydrocarbon hydroxylase**	0.505	0.057	—
Glutathione (nmol/mg protein)	—	333.	306.
Glutatione S-transferase towards***			
1-chloro-2,4-dinitrobenzene	—	—	2,370.
1,2-dichloro-4 nitrobenzene	—	—	34.
1,2-epoxy-3-(p-nitrophenoxy)propane	—	—	71.
UDP-Glucuronyltransferase towards****			
p-nitrophenol	33.0	40.8	19.4
phenolphthalein	—	16.8	9.5

* Values are representative *in vitro* measurements made during different investigations in the author's laboratory. Values can not be fairly compared to one another as they may be measurements performed on different days. Adapted from (87).

** nmole of 3-hydroxybenzo[a]pyrene/mg protein/min.

*** Rat liver glutathione S-transferase activity is not highly sex or pregnancy dependent.

**** Enzyme activity after detergent activation. Non-activated GT activity is about 1/10 these values.

pounds. However, for a large number of compounds this overall scheme can be useful in making qualitative and quantitative predictions about pesticide metabolism, kinetics and dynamics. The model also can predict how these factors will be influenced by alteration of various components of the scheme.

Computer based models of pesticide pharmacokinetics and pharmacodynamics could be constructed using a framework such as that presented in Figure 1. Naturally, substantial problems need to be solved in determining *in vivo* catalysis rates (Table 4), in using saturable enzyme kinetic parameters and in subcellular compartmentalization before an accurate computer model can be created. From such a model, mathematical predictions of additive, synergistic, and antagonistic interactions could be made prior to biological experimentation designed to test the predictions. As mathematical modeling capabilities are advancing at a faster rate than are improvements in toxicological testing, this approach will be even more attractive in the future than it is today. Nonetheless, substantial toxicological experimentation in animals will always be a public health necessity.

Footnote

For reasons of brevity, several important biotransformation pathways such as sulfation, acetylation, methylation, dehalogenation, dehydrohalogenation, dehydrogenation, nitro reduction, azo reduction, and epoxide hydration are omitted from this article. The hydrolysis of esters and their obvious central importance to pesticide toxicology have been described many times by various authors.

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